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COLOUR SIGNALS AND CHOICE BEHAVIOUR OF THE HONEYBEE (*APIS MELLIFERA LIGUSTICA*)

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Abstract—The colour choice of honeybees *Apis mellifera ligustica* was tested on a food source simulator providing sucrose solution in coloured feeders blue or yellow. No asymmetry for colour choice was detected in the present experimental set-up. Test of Runs showed that colour is used to switch from one group of coloured feeders to the other in a strategy that seems related to the probability of finding a reward on the same colour.

Key Word Index: Colour vision, honeybee, foraging strategy

INTRODUCTION

When foraging, honeybees localize the food sources by means of visual and olfactory cues (von Frisch, 1914, 1965; Butler, 1951). There is no agreement on the existence of spontaneous anticipation for some colours as reported by many authors (Oettingen-Spielberg, 1949; Butler, 1951; von Frisch, 1965; Martin, Personal Communication; see Menzel, 1985).

In this communication, the colour choice of foraging honeybees was tested on an experimental device providing sucrose solution in coloured feeders. A procedure was developed to avoid the effect of the pretraining on the so-called spontaneous colour choice, a problem encountered by some authors. In essence, our technique applies the "asymmetry" test of colour choice after well defined and balanced pretraining to detect any bias towards either of the two colours, blue and yellow.

MATERIALS AND METHODS

Apis mellifera ligustica from the apiary of S.A.D.A. (Argentine Beekeepers Society) in González Catán, Province of Buenos Aires (lat 34.6° south) were used. The hive was placed 60 m from the laboratory.

Apparatus

The food source simulator was placed in the laboratory on a table close to an open window. The simulator (Núñez, 1971) consisted of a 12-feeder unit, each feeder receiving sucrose solution (50% w/w) supplied by an automatic microburette of the Scholander type (Kirk, 1950), driven by a synchromotor (Fig. 1). The total flow rate supplied was 1.02 μl/min (each feeder received 1/12 of the total flow) and feeders were numbered and marked with a disc of coloured paper 5 cm in diameter on a grey background. With this flow supply, bees performed 196 ± 2.62 (mean ± SE; $n = 308$ visits) feeder inspections per visit. A detailed description of the simulator has already been published (Núñez, 1971). Colours used were blue and yellow, well discriminated by honeybees (Daumer, 1956; Rose and Menzel, 1981). The synchromotor was switched on when the bee arrived at the simulator, and turned off when she left to return to the hive.

Numbered feeders of the food source simulator were assigned to Group I (1, 2, 3, 5, 8, 12) and Group II (4, 6, 7, 9, 10, 11), following a random number series, to ensure aleatory distribution of places in the simulator when two colours were presented.

An infra-red heater maintained the temperature of the foraging area at approx. 24°C.

Experimental procedure

Bees were trained to find sugar solution at the simulator without colour marks. When they were foraging regularly at the simulator, one was selected for the measurements and identified with a coloured spot on the thorax. All other bees were caged, fed with sugar syrup and reserved for later use.

Four different experimental colour patterns on the simulator were used for training each bee during a series of successive visits:

(1) all feeders were marked with colour A (blue or yellow), as first training colour on the simulator;
(2) on Group I (or II) feeders, colour was changed to B (yellow or blue), i.e. a second alternative colour was presented on the simulator;
(3) all feeders were marked with the colour B (yellow or blue), as second training colour on the simulator;
(4) on Group II (or I) feeders, colour was changed to A (blue or yellow).

In a first training series, each pattern was presented during two successive visits, i.e. during visits 1 and 2, the bee found all feeders on the simulator marked with colour A; during visits 3 and 4, colours A and B;

during visits 5 and 6, colour B and finally, during visits 7 and 8, colours A and B.

In a second training series each pattern was presented during 9 successive visits, i.e. during visits 1–9, the bee found all feeders on the simulator marked with colour A, and so on.

Seven replications with one bee each, were carried out.

Behavioural parameters recorded were:

Visit-time: the time a bee spent at the food source simulator during a visit.

Non-visit-time: the time a bee spent away from the simulator between visits.

Feeder choice: approach of the flying bee to the feeders followed by landing and/or inspection for sugar solution.

Experiments were performed covering two different periods of nectar flow at natural food sources: Summer (December–March) with high nectar flow, and Autumn (March–May) with low nectar flow.

Statistics

Deviation from random distribution of inspections on two colours was tested with the "G-test" (Sokal and Rohlf, 1981).

Deviation from the random arrangement of sequences to the same colour was tested with the "Test of Runs" (Draper and Smith, 1966).

RESULTS

Position bias of choices for Groups I or II were tested through the 1st and 3rd experimental pattern, i.e. when all feeders on the simulator were marked with one and the same colour, yellow or blue. G-test results are presented in Table 1. No deviation from random distribution of inspections to Group I nor to Group II was observed.

Colour biased choice for the rewarded colour, yellow or blue, as a result of the 1st and 3rd patterns, was tested with 2nd and 4th experimental patterns, i.e. when feeders of one Group (I or II) were marked with the alternative colour, bees finding at the simulator 6 feeders identified with yellow and 6 with blue

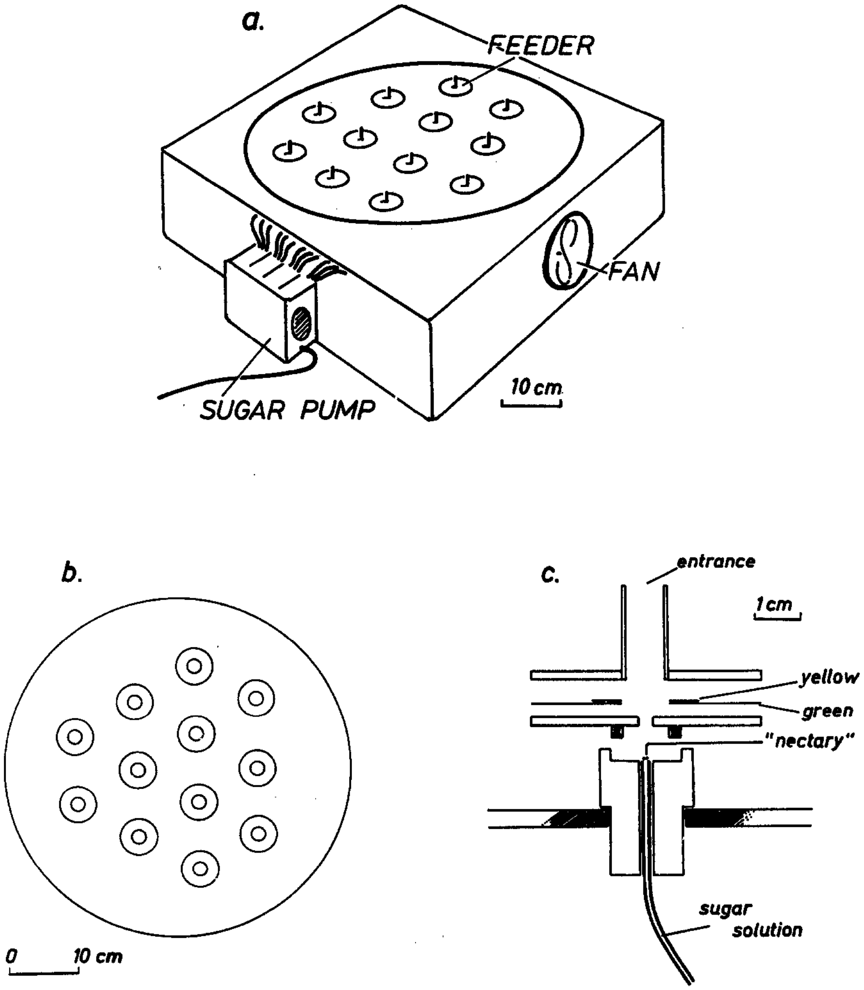

Fig. 1. Food Source Simulator. (a) general view; (b) upper view of the 12 feeders; (c) detail of a feeder.

Table 1

Pattern	First training series visit	G	P	Second training series visit	G	P
1st	1	0.35	NS	1	0.03	NS
	2	0.00	NS	2	0.25	NS
2nd	3	10.36	< 0.005	10	11.56	< 0.001
	4	0.67	NS	11	0.27	NS
3rd	5	0.71	NS	19	0.74	NS
	6	0.38	NS	20	0.72	NS
4th	7	0.08	NS	28	0.05	NS
	8	0.67	NS	29	0.005	NS

For the second training series, only the two first visits of each pattern are presented. "G" values for all other visits were NS.

colour. G-test results for colour-biased behaviour show that during the first visit to the new pattern with two colours, the choice of the colour previously rewarded proved significantly greater. Nevertheless, colour choice asymmetry persisted only during the first third of the visit.

No differences in the fading rate of choice asymmetry were observed when comparing results of first and second training series, i.e. after 2 or 9 rewarded visits. During the 4th pattern no deviation at all from random distribution was recorded.

To test "floral constancy" of bees postulated by many authors (von Frisch, 1914, 1965), we analysed records after the "Test of Runs". This test evaluates whether the number of runs deviates from random arrangement. In other words, if bees make sequences of inspections related to colour in an unusual pattern, i.e. if they follow or alternate colours of feeders inspected, this will be apparent through a lower or higher number of runs, respectively.

Results obtained by comparing the pooled data from 1st and 3rd patterns vs 2nd and 4th patterns (one colour vs two colours on the simulator) are presented in Table 2(a) for the first and in 2(b) for the second training series. Thereafter bees showed a colour-biased behaviour in the number of runs performed, when two colours were presented in the simulator. Whereas in the 1st and 3rd patterns (control = one colour in the simulator), the number of runs deviated from random arrangement in only 9 visits out of 126 recorded, the same occurred in 107 visits out of 126 recorded in the 2nd and 4th patterns (test = two colours in the simulator). This difference is highly significant ($P < 0.001$, G-test).

Mean run length of inspections (total number of inspections/number of runs) was greater than expected for a random choice during the first visit of the 2nd pattern in both training series: the bees "followed the colour A". On all other visits in both training sessions, the mean run length was significantly lower than expected for a random choice (mean run length for 3rd pattern = 1.88 ± 0.06 vs 4th pattern = 1.73 ± 0.03; $t = 6.3245$, $P < 0.001$). Results are presented in Table 3.

Table 3

Pattern	First training series visit	MRL ± SD	Second training series visit	MRL ± SD
2	3	2.37 ± 0.32	10	2.75 ± 1.01
	4	1.77 ± 0.17	11	1.57 ± 0.14
4	7	1.72 ± 0.04	28	1.70 ± 0.04
	8	1.74 ± 0.06	29	1.74 ± 0.09

MRL: Mean run length = total number of inspections/number of runs.

DISCUSSION

Bees show no asymmetry for colour choice in the present experimental set-up (Table 1). This lack of asymmetry for colour choices was independent of the season of the year, time of day, and sequence of colour tested. After extensive conditioning to one colour in the absence of others, choices showed a bias to this colour only during the first visit to the 2nd pattern (two colours—Table 1). This biased colour behaviour was compensated after a few rewarded inspections to the new colour presented during the 2nd pattern. This means that bees reverse their choice behaviour quickly if they are rewarded at the new colour.

Statistical analysis of the number of runs to colours showed that colour is continuously used as an orientation cue during foraging trips at the food source simulator. This is depicted in Table 2(a) and (b) where a significant deviation of the number of runs from random arrangement for the two colour patterns was evidenced by a greater number of visits as compared with the control (one colour pattern). On the other hand, position information of the feeders was not used by the foraging bee on the simulator. This is also illustrated in Table 2(a) and (b) where, in the control (one colour), a significant number of visits failed to deviate in its number of runs from a random arrangement in relation to Groups I or II.

Taking into account the run length, the learned colour was followed through longer runs than random expected during the first visit to the 2nd pattern. Thereafter, choices showed a significant bias to shorter runs than expected from random distribution (Table 3). However, as the changes from "following the trained colour" to "alternate" occurred after a few inspections and with dissimilar delay in each bee,

Table 2

Test of runs	1st and 3rd pattern (control)	2nd and 4th pattern (test)	G (test vs control)	P
(a) first training series				
<0.005	1	27	31.57	<0.001
NS	27	1	31.57	< 0.001
Total visits	28	28		
(b) Second training series				
< 0.005	9	107	97.51	< 0.001
NS	117	19	78.53	< 0.001
Total visits	126	126		

mean run length for the first visit showed high variability and was not statistically different from random arrangement. This result suggests that colour information is used to switch from one group of coloured feeders to the other in a strategy that seems related to the probability of finding a reward on the same colour. Without use of position information, each further inspection to the same colour would be expected to be less rewarded.

Our results support the conclusion that colour is a cue to make decisions as one would expect from the "floral constancy strategy". Under natural conditions, different flowers may need different motor patterns to exploit nectar and/or pollen. Such flowers are very likely to be marked with different colours. Colour constancy would thus reflect a strategy to optimise the handling procedure by avoiding the complication that the animal would have to associate different motor patterns with colours at the same time. Foraging would be accomplished with maximal efficiency by "working on line", i.e. by following one flower type. Whether this strategy is used in all biotopes by all bee species remains to be determined.

Bumblebees of any one species forage for pollen and/or nectar from a large variety of flowers and inviduals have limited foraging repertoires at one time. Unspecialized individuals seem to be less successful in extracting nectar and/or pollen from highly rewarding flowers (Heinrich, 1976). Bumblebees species are floral generalists but specialists as individuals. However, the finding that in tropical rain forests different species of bees seem to be polylectic, i.e. they habitually inspect a variety of flower species during one and the same foraging trip, and that the African *Apis mellifera scutellaris* in South America evinces a high degree of polylectic behaviour during its trips (Núñez, unpublished observations) show that other strategies are possible.

Spontaneous choice of colours at the feeding place is practically impossible to test experimentally because the animal has to be motivated by feeding to search at a certain location for food, and any feeding involves the fast learning of visual and other cues close to the feeding place, since bees generalize effectively from one visual cue to the other (see Menzel, 1985 for review). It is to be expected that any pretraining would have a strong impact on the colour choice even if no obvious colour marks or "neutral" marks (grey, white or black) were used. This was actually observed in unpublished experiments by Núñez and Menzel. We thus used the test of asymmetry in successive training experiments to search for any anticipation of the bee to use blue or yellow more readily as a colour mark for feeding. Since we failed to find any asymmetry, it may be concluded that both blue and yellow are of similar prominence as colour marks, which would also indicate the lack of initial preference for one of the two colours before training. However, there is also the possibility that learning of colours at the feeding place is such a strong behaviour that it overrides any weak spontaneous preference.

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